



Certificate of Analysis

Standard Reference Material[®] 2395

Human Y-Chromosome DNA Profiling Standard

This Standard Reference Material is intended primarily for use in the standardization of forensic and paternity quality assurance procedures for polymerase chain reaction (PCR)-based genetic testing and for instructional law enforcement or non-clinical research purposes that involve the human Y-chromosome. In addition, SRM 2395 may be used to standardize nomenclature for the field of genetic genealogy. This SRM can also be used for quality assurance when assigning values to in-house control materials. It is not intended for any human or animal clinical diagnostic use. Additional information on each Y-chromosome marker can be found at a NIST-sponsored database on the Internet: <http://www.cstl.nist.gov/biotech/strbase>.

This SRM is composed of well-characterized human genomic deoxyribonucleic acid (DNA) in liquid form. A unit of SRM 2395 consists of six frozen components (A through F) packaged in one box. Each component contains 50 μ l of DNA at a concentration of approximately 1 ng/ μ L. There are five male samples and one female sample in this SRM. See Table 1 for a complete listing of the components.

Certified Values: A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The SRM is certified for genetic loci on the human Y-chromosome [2–13]. Genetic types for loci were certified through DNA quantitation, sequencing, interlaboratory testing, and typing methodologies. Table 1 provides the quantitative PCR (qPCR) results from multiple analyses of two SRM 2395 units obtained from the NIST Measurement Service Division and one unit from the NIST Biochemical Science Division stored at -20°C . Table 2 lists the certified genetic types in normal font for 41 different Y-chromosome short tandem repeat (Y-STR) markers. All certified types have been confirmed through DNA sequencing of the alleles for each of the 5 male components.

Reference Values: A NIST Reference Value is a best estimate of the true value provided by NIST where all known or suspected sources of bias have not been fully investigated by NIST [1]. Table 2 also includes three reference genetic types in bold font for different Y-STR markers. Reference alleles have been typed but NOT fully confirmed through DNA sequencing.

Informational Values: An information value is considered to be a value that will be of interest and use to the SRM user, but for which insufficient information is available to assess adequately the uncertainty associated with the value, or a value derived from a limited number of analyses [1]. Table 3 describes informational values for three additional Y-STR loci that have been typed but not yet sequenced. Table 4 lists the genetic types for 42 different Y-chromosome single nucleotide polymorphisms (Y-SNPs) determined by allele-specific hybridization. Figure 1 displays results from the quadruplicated Y-STR DYS464.

Expiration of Certification: The certification of SRM 2395 is valid, within the measurement uncertainties specified, until **31 December 2013**, provided the SRM is handled and stored in accordance with the instructions given in this certification (see “Instructions for Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the technical activities leading to certification were under the leadership of M.C. Kline and J.M. Butler of the NIST Biochemical Science Division.

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Analytical determination and technical measurements leading to the certification of this SRM were performed by staff of the NIST Biochemical Science Division: M.C. Kline and J.W. Redman prepared the samples; J.M. Butler, R. Schoske, and P.M. Vallone initially evaluated the components using selected PCR amplification assays and kits to determine the certified genotypes; A.E. Decker and M.C. Kline evaluated the stability of the components in selected quantitative PCR (qPCR) assays; A.E. Decker performed a concordance evaluation of the components by comparing the initial genotypes with recently obtained genotypes using Y-STR PCR amplification kits; and A.E. Decker performed sequencing and genotyping of 21 Y-STR loci to determine certified and reference values.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

The preparation of this SRM was supported in part by the National Institute of Justice, U.S. Department of Justice.

NOTICE AND WARNINGS TO USER

Storage: All six components must be stored frozen at a temperature of $-20\text{ }^{\circ}\text{C}$. **DO NOT** use a self-defrosting freezer because periodic cycling of temperatures may cause a shortened shelf-life of this SRM.

Handling: SRM 2395 IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE THE SRM 2395 COMPONENTS AS BIOSAFETY LEVEL 1 MATERIALS CAPABLE OF TRANSMITTING INFECTIOUS DISEASE [14]. SRM 2395 components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

INSTRUCTIONS FOR USE

Sample aliquots for analysis should be withdrawn immediately after opening the vials and should be processed without delay for the certified values to be applicable. Let samples warm up to laboratory ambient temperature for 2 hours before use.

SOURCE AND ANALYSIS¹

Source of Material: Genomic DNA components were extracted from whole blood obtained from Millennium Biotech, Inc. (Ft. Lauderdale, FL).

Interlaboratory Analysis: The STR values for this SRM represent the pooled results from analyses performed at NIST, ReliaGene Technologies, Inc. (New Orleans, LA), OligoTrail LLC (Evanston, IL), the Forensic Laboratory for DNA Research at Leiden University Medical Center (Leiden, The Netherlands), Sorenson Molecular Genealogy Foundation (Salt Lake City, UT) and Family Tree DNA (Houston, TX).

Additional Information: Further characterization of SRM 2395 components through qPCR and DNA sequencing is summarized on the NIST-sponsored Short Tandem Repeat DNA Internet DataBase (STRBase) and available at <http://www.cstl.nist.gov/biotech/strbase/srm2395.htm>.

¹Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Commercial kits used to obtain typing results used at NIST:

PowerPlex® Y (Promega):

DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439

AmpFISTR® Yfiler™ PCR Amplification Kit (Applied Biosystems):

DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, Y-GATA-H4

Signet™ Y-SNP Identification System (Marligen Biosciences, Ijamsville, MD):

42 Y-SNPs listed in Table 4

Table 1. DNA Concentration Ranges of SRM 2395 Components Determined by Multiple qPCR Analysis Runs^(a)

Component	Description	DNA Concentration Range (ng/μL)
A	Male Genomic DNA 1	0.87 – 2.06
B	Male Genomic DNA 2	0.85 – 1.59
C	Male Genomic DNA 3	1.07 – 1.66
D	Male Genomic DNA 4	0.99 – 1.43
E	Male Genomic DNA 5	1.02 – 1.55
F ^(b)	Female Genomic DNA	0.96 – 1.36

^(a) DNA concentrations given are not intended for use as concentration standards.

^(b) Component F is to serve as a negative control for Y-chromosome specific assays.

Table 2. Certified and Reference Values for the 41 Y-STR Markers in SRM 2395 Components A–F^(a)

Y-STR Marker	A	B	C	D	E	F
DYS19	14	14	16	15	17	0
DYS385a/b	12,15	14,17	17,20	14,15	13,15	0
DYS388	12	15	12	12	13	0
DYS389I	13	13	14	12	14	0
DYS389II	29	28	32	28	31	0
DYS390	25	23	21	22	24	0
DYS391	11	11	12	10	10	0
DYS392	13	11	11	11	12	0
DYS393	13	12	13	14	14	0
DYS426	12	11	11	11	11	0
DYS435	12	11	11	11	11	0
DYS436	12	12	12	12	12	0
DYS437	15	14	14	16	14	0
DYS438	12	9	11	11	10	0
DYS439	12	12	11	11	11	0
DYS447	24	25 ^(b)	25 ^(b)	23	26	0
DYS448	19 ^(c)	21 ^(c)	21 ^(c)	21 ^(c)	20 ^(c)	0
DYS449	28	32	30	28	27	0
DYS456	15	15	15	15	15	0
DYS458	16	15	17	16	16	0
DYS460	11	10	9	11	11	0
DYS461	12	13	13	11	12	0
DYS481	22	23	28	23	28	0
DYS635	23	21	23	21	21	0
Y-GATA-H4	12 ^(d)	12 ^(d)	12 ^(d)	12 ^(d)	11 ^(d)	0
DYS492	12	12	11	11	12	0
DYS522	10	11	10	12	12	0
DYS527	21,23	22	17,20	15,21	22,23	0
DYS532	15	11	12	15	9	0
DYS534	15	15	15	14	14	0
DYS570	17	18	18	17	18	0
DYS572	11	11	9	9	11	0
DYS576	18	16	17	18	17	0
DYS607	19	19	19	17	18	0
DYS650	18	18	16	18	24	0
DYS652	24	25	25	23	26	0
DYS709	13	16	15	17	16	0
DYS710	36	34.2	35.2	33.2	31	0
DYS712	23	22.3	21	26	19	0
DYS715	24	21	22	23	23	0
DYS717	17	17	17	20	14	0

^(a) These values have all been confirmed through DNA sequencing at NIST (see STRBase for repeat motif information) except for the three reference values in bold.

^(b) Components B and C for DYS447 are listed as 25 repeats. However, B contains 9-1-8-1-6 while C is 7-1-8-1-8 in terms of [TAATA]-[TAAAA]-[TAATA]-[TAAAA]-[TAATA] repeats (see STRBase).

^(c) Three different nomenclatures have been published for DYS448 [10]. The one listed here follows Redd *et al* [6].

^(d) The H4 nomenclature follows Butler *et al.* [5]; also see reference 16.

Table 3. Informational Values for Additional Y-STR Loci in SRM 2395 Components A–F

Component	Name	DYS450	DYS464 a/b/c/d (expanded)	DYS464 a/b/c/d (conservative)	YCAII a/b
A	Male 1	10	14-15-15-17	14-15-17	19-23
B	Male 2	10	12-13-13-17	12-13-17	19-22
C	Male 3	8	13-16-16-18	13-16-18	19-19
D	Male 4	9	13-13-14-14	13-14	20-20
E	Male 5	10	11-14-14-15	11-14-15	19-21
F	Female				

Information in Table 3 has not been fully confirmed through direct DNA sequencing of the SRM components. DYS464 is quadruplicated on the Y-chromosome and can produce up to four separate peaks (see Figure 1). Both expanded and conservative methods for calling DYS464 alleles are listed (see references 6 and 11).

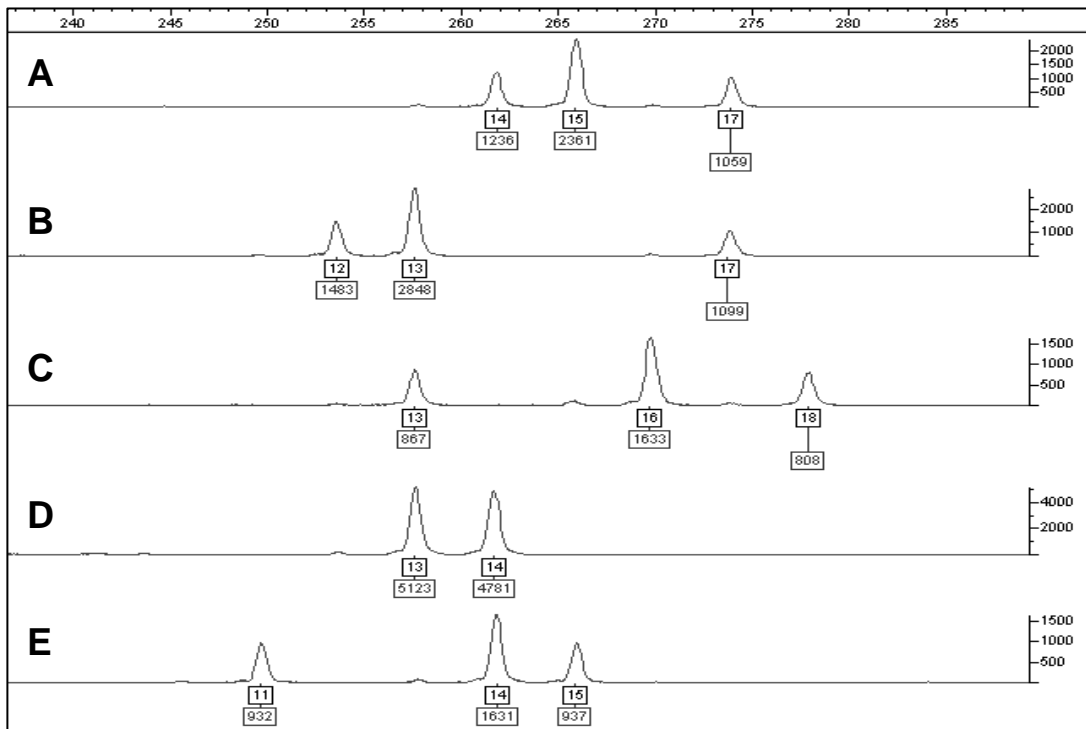


Figure 1. Allele calls (top label under peak) and peak heights (bottom label) for the multi-copy Y-STR DYS464, which is quadruplicated on the Y-chromosome.

Table 4. Information Values for 42 Y-SNP Loci in SRM 2395 Components A–F^(a,b)

Y-SNP Marker/ Haplogroup	A	B	C	D	E	F
Amelogenin	X,Y	X,Y	X,Y	X,Y	X,Y	X,X
DYS391 C/G	C	C	G	C	C	–
M119 A/C	A	A	A	A	A	–
M11 A/G	A	A	A	A	A	–
M124 C/T	C	C	C	C	C	–
M130 C/T	C	C	C	C	C	–
M146 A/C	A	A	A	A	A	–
M150 C/T	C	C	C	C	C	–
M153 T/A	T	T	T	T	T	–
M157 A/C	A	A	A	A	A	–
M168 C/T	T	T	T	T	T	–
M170 A/C	A	A	A	A	C	–
M172 T/G	T	G	T	T	T	–
M174 T/C	T	T	T	T	T	–
M175 +/-	+	+	+	+	+	–
M18 -/+	–	–	–	–	–	–
M182 C/T	C	C	C	C	C	–
M201 G/T	G	G	G	T	G	–
M207 A/G	G	A	A	A	A	–
M2 A/G	A	A	G	A	A	–
M31 G/C	G	G	G	G	G	–
M32 T/C	T	T	T	T	T	–
M33 A/C	A	A	A	A	A	–
M35 G/C	G	G	G	G	G	–
M37 C/T	C	C	C	C	C	–
M3 C/T	C	C	C	C	C	–
M42 A/T	T	T	T	T	T	–
M45 G/A	A	G	G	G	G	–
M52 A/C	A	A	A	A	A	–
M5 C/T	C	C	C	C	C	–
M60 -/+	–	–	–	–	–	–
M75 G/A	G	G	G	G	G	–
M87 T/C	T	T	T	T	T	–
M89 C/T	T	T	C	T	T	–
M94 C/A	A	A	A	A	A	–
M95 C/T	C	C	C	C	C	–
P25 C/A	A	C	C	C	C	–
P3 C/T	C	C	C	C	C	–
P4 G/A	G	G	G	G	G	–
SRY10831 A/G	G	G	G	G	G	–
SRY465 C/T	C	C	C	C	C	–
SRY9138 C/T	C	C	C	C	C	–
Tat T/C	T	T	T	T	T	–
YCC Haplogroup	R1b	J2	E3a	G	I	–

^(a) SRM components were typed through allele-specific hybridization using the Signet Y-SNP Identification System (Marligen Biosciences, Ijamsville, MD) [12].

^(b) Derived alleles are shaded to designate Y-Chromosome Consortium (YCC) haplogroups [13].

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Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: Telephone (301) 975-6776; fax (301) 926-4751, e-mail srminfo@nist.gov, or via the Internet at <http://www.nist.gov/srm>.